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09/719088
PCT/AU99/00523

AU99/00523

REC'D 03 AUG 1999

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AUSTRALIA

Patents Act 1990

GARVAN INSTITUTE OF MEDICAL RESEARCH

PROVISIONAL SPECIFICATION

Invention Title:

NPY-Y7 Receptor Gene

The invention is described in the following statement:

Field of Invention:

The present invention relates to isolated DNA molecules which encode a novel Y-receptor designated NPY-Y7. In addition, the present invention relates to the use of these molecules in the production of NPY-Y7
5 receptors using recombinant technology and to methods of screening and testing compounds for agonist or antagonist activity.

Background of the Invention:

Neuropeptide Y (NPY) forms a family (called the pancreatic
10 polypeptide family) together with pancreatic polypeptide (PP) and peptide YY (PYY), which all consist of 36 amino acids and possess a common tertiary structure. Neuropeptide Y (NPY) receptors, members of the G protein-coupled receptor superfamily, when activated influence a diverse range of important physiological parameters, including effects on psychomotor
15 activity, central endocrine secretion, anxiety, reproduction, vasoactive effects on the cardiovascular system and strongly stimulates food consumption. Specific agonists and antagonists of NPY are therefore likely to be of substantial benefit for therapy of a wide range of clinical disorders. As NPY possess a compact tertiary structure and different parts of the molecule are
20 required for interaction with different subtypes of the receptor, the logical developments of both agonists and antagonists is critically dependent upon the availability and knowledge of specific receptor structure.

It is presently known that NPY binds specifically to at least six receptors; Y1, Y2, Y3, Y4, Y5 (or "atypical Y1") and Y6. While it has been
25 demonstrated that NPY receptors couple to the adenylate cyclase second messenger system, it remains probable that additional NPY receptor subtypes exist since there is evidence that phosphatidylinositol turnover, cations, and arachidonic acid may also function as second messengers for NPY.

Since NPY agonists and antagonists may have commercial value as,
30 for example, potential anti-hypertensive agents, cardiovascular drugs, neuronal growth factors, anti-psychotics, anti-obesity and anti-diabetic agents, the ability to produce NPY receptors by recombinant DNA technology would be advantageous. To this end, DNA molecules encoding Y1, Y2, Y4, Y5 and Y6 have previously been isolated.

The present inventors have now isolated novel DNA molecules encoding the human and murine NPY-Y7 receptors.

Summary of the Invention:

5 Thus, in a first aspect, the present invention provides an isolated DNA molecule encoding an NPY-Y7 or a functionally equivalent fragment thereof.

 Preferably, the encoded NPY-Y7 receptor is characterised by the N-terminal amino acid sequence:

10 MX₁X₂MX₃EKWDX₄NSSE,

 wherein X₁, X₂, X₃ and X₄ are selected from codable amino acids but, more preferably, X₁ is selected from Phe and Ser, X₂ is selected from Ile and Thr, X₃ is selected from Asn and Ser, and X₄ is selected from Thr and Ser.

 More preferably, the isolated DNA molecule encodes a human NPY-
15 Y7 receptor of about 408 amino acids or a murine NPY-Y7 receptor of about 405 amino acids.

 Most preferably, the isolated DNA molecule encodes a human NPY-Y7 receptor having an amino acid sequence substantially corresponding to that shown at Figure 1 or a murine NPY-Y7 receptor having an amino acid
20 sequence substantially corresponding to that shown at Figure 2.

 The nucleotide sequence of a DNA molecule in accordance with the first aspect may comprise a nucleotide sequence substantially corresponding or, at least, showing >90% (more preferably >95%) identity to that shown at nucleotides 369 to 1592 or nucleotides 1 to 1903 of Figure 1 or any portion
25 thereof encoding a functionally equivalent NPY-Y7 receptor fragment.

 The isolated DNA molecule may be incorporated into plasmids or expression vectors (including viral vectors), which may then be introduced into suitable bacterial, yeast, insect and mammalian host cells. Such host cells may be used to express the NPY-Y7 receptor encoded by the isolated
30 DNA molecule.

 Accordingly, in a second aspect, the present invention provides a mammalian, insect, yeast or bacterial host cell transformed with the DNA molecule of the first aspect.

 In a third aspect, the present invention provides a method of
35 producing NPY-Y7 receptors or functionally equivalent fragments thereof, comprising culturing the host cell of the second aspect under conditions

enabling the expression of the DNA molecule and optionally recovering the NPY-Y7 receptors or functionally equivalent fragments thereof.

Preferably, the host cell is mammalian or of insect origin. Where the cell is mammalian, it is presently preferred that it be a Chinese hamster
5 ovary (CHO) cell, monkey kidney (COS) cell or human embryonic kidney 293 cell. Where the cell is of insect origin, it is presently preferred that it be an insect Sf9 cell.

In a preferred embodiment, the NPY-Y7 receptors or fragments thereof are expressed onto the surface of the host cell.

10 The DNA molecules of the present invention encode a NPY receptor which may be of interest both clinically and commercially as it is expressed in many regions of the body and neuropeptides of the NPY family affect a wide number of systems.

By using the nucleic acid molecules of the present invention it is
15 possible to obtain NPY-Y7 receptor protein or fragments thereof in a substantially pure form.

Accordingly, in a fourth aspect, the present invention provides a NPY-Y7 receptor or a functionally equivalent fragment of said receptor, in a substantially pure form.

20 Preferably, the NPY-Y7 receptor of the fourth aspect is characterised by the N-terminal amino acid sequence:

$MX_1X_2MX_3EKWDX_4NSSE$,

wherein X_1 , X_2 , X_3 and X_4 are selected from codable amino acids but, more preferably, X_1 is selected from Phe and Ser, X_2 is selected from Ile and Thr,
25 X_3 is selected from Asn and Ser, and X_4 is selected from Thr and Ser.

More preferably, the purified NPY-Y7 receptor has an amino acid sequence substantially corresponding to that shown in Figure 1 or 2.

In a fifth aspect, the present invention provides an antibody capable of specifically binding to the NPY-Y7 receptor of the fourth aspect.

30 In a sixth aspect, the present invention provides a non-human animal transformed with a DNA molecule according to the first aspect of the present invention.

In a seventh aspect, the present invention provides a method for detecting agonist or antagonist agents of an NPY-Y7 receptor, comprising
35 contacting an NPY-Y7 receptor, functionally equivalent fragment thereof or a cell transfected with and expressing the DNA molecule of the first aspect,

with a test agent under conditions enabling the activation of a NPY-Y7 receptor, and detecting an increase or decrease in activity of the NPY-Y7 receptor or functionally equivalent fragment thereof.

5 An increase or decrease in activity of the receptor or functionally equivalent fragment thereof may be detected by measuring changes in cAMP production, Ca^{2+} levels or IP3 turnover after activating the receptor or fragment with specific agonist or antagonist agents.

10 In a further aspect, the present invention provides a nucleic acid probe comprising a nucleotide sequence of 10 or more nucleotides capable of specifically hybridising to a unique sequence within the DNA molecule of the first aspect.

15 In a still further aspect, the present invention provides an antisense nucleic acid molecule comprising a nucleotide sequence capable of specifically hybridising to an mRNA molecule which encodes a NPY-Y7 receptor so as to prevent translation of the mRNA molecule.

Such antisense nucleic acid molecules may include a ribozyme region to catalytically inactivate mRNA to which it is hybridised.

20 The DNA molecule of the first aspect of the invention may be a dominant negative mutant which encodes a gene product causing an altered phenotype by, for example, reducing or eliminating the activity of endogenous NPY-Y7 receptors.

25 The term "substantially corresponding" as used herein in relation to amino acid sequences is intended to encompass minor variations in the amino acid sequences which do not result in a decrease in biological activity of the NPY-Y7 receptor. These variations may include conservative amino acid substitutions. The substitutions envisaged are:-

G, A, V, I, L, M; D, E; N, Q; S, T; K, R, H; F, Y, W, H; and P, N α -alkalamino acids.

30 The term "substantially corresponding" as used herein in relation to nucleotide sequences is intended to encompass minor variations in the nucleotide sequences which due to degeneracy in the DNA code do not result in a change in the encoded protein. Further, this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not
35 result in a decrease in biological activity of the encoded protein.

The term "functionally equivalent fragment/s" as used herein is intended to refer to fragments of the NPY-Y7 receptor that exhibit binding specificity and activity that is substantially equivalent to the NPY-Y7 receptor from which it/they is/are derived.

5 The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step, component or feature or group of steps, components or features with or without the inclusion of a further step, component or feature or group of steps, components or features.

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Detailed Disclosure of the Invention:

Human NPY-Y7 cDNA

Human amygdala and testis cDNA libraries (Stratagene) were screened under low stringency conditions with a 401 bp ³²P-labelled fragment (corresponding to nucleotides 507 to 908 of Figure 1) originated from a human fetal brain EST clone (GenBank AA449919). Two overlapping cDNA clones were obtained from the screen. The combined nucleotide sequence (hy7) of the clones is shown at Figure 1 and encodes a protein of 408 amino acids.

20

Sequence comparison with other G protein coupled receptors identified neuropeptide Y receptors as the most closely related group with approximately 32% amino acid sequence identity to the Y1 receptor subtype (Figure 3). Further, *in situ* hybridisation studies of rat brain sections has identified a NPY-Y7 mRNA distribution (expression was found to occur in the amygdala, the CA3 region of the hippocampus and the piriform cortex) which is consistent with the expression of other NPY-receptor subtypes (Blomquist, A.G., and Herzog, H., TINS 20(7), 1997) and is in agreement with the suggestions of the existence of further Y-receptor family members. This mRNA distribution suggests important functions for the NPY-Y7 receptor in the regulation of the circadian rhythm, anxiety and metabolic status.

25

Radio-ligand binding experiments has shown that the protein encoded by the hy7 cDNA shows highest affinity for human PYY. These experiments were conducted using COS-6 or HEK (293) cells transiently expressing recombinant Y7 receptor protein. The radio-ligand binding (Herzog, H. et al., Proc. Natl. Acad. Sci. USA 89:5794-5798, 1992) suggests that the NPY-Y7 receptor has a pharmacology similar to the Y2 receptor

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(Rose, P., J. Biol. Chem. 270:22661-22664, 1995). The rank of potency for the Y7 receptor is:

PYY>NPY>[2-36]PYY>[3-36]NPY>[13-36]NPY>>(Leu31, Pro34)NPY>PP.

Chromosomal Localisation of the Human Y7 gene

5 Screening of a medium resolution Stanford G3 panel of 83 clones was performed to further refine the map position of the hy7 gene. PCR amplification was carried out on this panel using primers hy7-A (5'GGATGGCCATTTGGAAAC3') and hy7-B (5'CCAATCCTTCCATACATG3'), corresponding to nucleotides 507-524 and 890-907 of the hy7 cDNA, respectively. The analysis indicated that the hy7 gene is most closely associated with the marker SHGC-418 on the long arm of chromosome 4. This map location is defined by markers AFM191xh2 and AFM347ZH1. Assessment of the flanking markers using the Whitehead/MIT STS-Based Map of the Human Genome)(http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map) in conjunction with The Genome Directory (Adams, M.D., et al. (1995) Nature 377 Suppl.) identifies 4q21.3 as the most likely position of the hy7 gene.

Mouse Y7 genomic DNA

20 Using a ³²P-labelled fragment of the hy7 cDNA a mouse genomic BAC library (Genome Systems) was screened. A clone encoding the entire gene of the mouse equivalent to hy7 was isolated (Figure 2). The gene covers approximately 12 kb and is divided by two introns into three exons (Figure 5).

Pharmacological characterisation

25 pcDNA3.1-hy7 cDNA was transiently transfected into the COSm6 cell line using FUGENE and 5mg of DNA/106 cells. The COSm6 cells were grown in Dulbecco's modified Eagles medium supplemented with 2mM glutamine and 10% fetal calf serum, in 5% CO₂ at 37°C. Membranes were harvested with COSm6 cells 72hr post-transfection. Adherent cells were washed twice in ice-cold phosphate buffered saline and lysed using a glass homogeniser in ice-cold hypotonic buffer (50mM Tris-HCl, pH 7.4, 0.1% bacitracin). Membranes were pelleted by high speed centrifugation (30,000 x g, 15min, 4°C), homogenised again in ice-cold hypotonic buffer and collected again by high speed centrifugation (30,000 x g, 15min, 4°C). The final membrane pellet was resuspended into 1ml of ice-cold binding buffer (50mM Tris-HCl, pH7.4, 10mM NaCl, 5mM MgCl₂, 2.5mM CaCl₂, 0.1% bacitracin, 0.1%

bovine serum albumin. Membrane suspensions were diluted in binding buffer to yield membrane protein concentrations of 0.05mg/ml. Under these conditions non-specific binding of [125 I]PYY to membranes was less than 10%. [125 I]PYY and unlabelled peptide competitors were also diluted to the required concentrations in binding buffer. Samples were prepared by mixing 50ml binding buffer, unlabelled peptide or binding buffer (50ml), [125 I]PYY (50mM, 50ml) and membrane suspension (100ml). Samples were incubated at room temperature for 2hr. Incubations were terminated by centrifugation (4min) and pellets collected. Radioactivity was measured for 1min in a g counter.

Brief description of the accompanying Figures:

Figure 1 provides the nucleotide sequence of a cDNA encoding the human NPY-Y7 receptor and includes the predicted amino acid sequence.

Figure 2 provides the nucleotide sequence of a genomic DNA encoding the murine NPY-Y7 receptor and includes the predicted amino acid sequence.

Figure 3 shows the degree of identity between the predicted amino acid sequence of the human Y1, Y2 and Y7 receptors.

Figure 4 shows the degree of identity between the predicted amino acid sequence of the human and murine NPY-Y7 receptors

Figure 5 provides a schematic diagram of the murine NPY-Y7 receptor gene. The gene covers approximately 12 kb and consists of three exons.

Figure 6 provides a graph showing the inhibition of human [125 I]PYY binding with various NPY-related peptides on human Y7 membranes. The results were obtained through competitive displacement of [125 I]PYY on membranes of COSm6 cells transiently expressing human Y7 receptors. Membranes were incubated with [125 I]PYY (50pM) and increasing concentrations of peptide competitors. Data are representative of a single experiment with each point measured in triplicate.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to
5 be considered in all respects as illustrative and not restrictive.

Dated this twenty-ninth day of June 1998

GARVAN INSTITUTE OF MEDICAL
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Patent Attorneys for the Applicant:

F B RICE & CO

FIGURE 1

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* * * * * 70
CTCGAGATCCATTGTGCTCTAAAGGCCTCCTGAGTAGCTGGGACTACAGGCGCCCGCCACCACGCCTGGC
* * * * * 140
TAATTTTTTTGTATTTTTAGTAGGGACGGCGTTTCACTGTGTTAGCCAGATGGTCTCCATCTCCCGACCT
* * * * * 210
CGTGATCCACCCACCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGACCGCGCCCGGCCAATTTCC
* * * * * 280
TTTCTTAGTTGCCTCTGCCCACCTCTTCTCTTCTGCTTCCATATTACAGGTTTCCTCAGTTGCGAAATTA
* * * * * 350
GGATGTTAATTATAGCTTTTGACATACAAGAAACATCAAAAAGATTGAATGTCTTAATAAGAGTGAAGCA
* * * * * 420
TGTAGATCAGTGACTGCTATGTTTCATCATGAATGAGAAATGGGACACAAACTCTTCAGAAAACCTGGCATC
M F I M N E K W D T N S S E N W H
* * * * * 490
CCATCTGGAATGTCAATGACACAAAGCATCATCTGTA CTAGATATTAATATTACCTATGTGA ACTACTA
P I W N V N D T K H H L Y S D I N I T Y V N Y Y
* * * * * 560
TCTTCACCAGCCTCAAGTGGCAGCAATCTTCATTATTTCTACTTTCTGATCTTCTTTTGTGCATGATG
L H Q P Q V A A I F I I S Y F L I F F L C M M
* * * * * 630
GGAAATACTGTGGTTTGCTTTATTGTAATGAGGAACAAACATATGCACACAGTCACTAATCTCTTCATCT
G N T V V C F I V M R N K H M H T V T N L F I
* * * * * 700
TAAACCTGGCCATAAGTGATTACTAGTTGGCATATTCTGCATGCCTATAACACTGCTGGACAATATTAT
L N L A I S D L L V G I F C M P I T L L D N I I
* * * * * 770
AGCAGGATGGCCATTTGGAAACACGATGTGCAAGATCAGTGGATTGGTCCAGGGAATATCTGTGCGAGCT
A G W P F G N T M C K I S G L V Q G I S V A A
* * * * * 840
TCAGTCTTTACGTTAGTTGCAATTGCTGTAGATAGGTTCCAGTGTGTGGTCTACCCTTTTAAACCAAAGC
S V F T L V A I A V D R F Q C V V Y P F K P K
* * * * * 910
TCACTATCAAGACAGCGTTTGTGTCATTATTATGATCATCTGGGTCCTAGCCATCACCATTATGTCTCCATC
L T I K T A F V I I M I I W V L A I T I M S P S
* * * * * 980
TGCAGTAATGTTACATGTGCAAGAAGAAAAATATTACCGAGTGAGACTCAACTCCCAGAATAAAACCACT
A V M L H V Q E E K Y Y R V R L N S Q N K T S
* * * * * 1050
CCAGTCTACTGGTGCCGGAAGACTGGCCAAATCAGGAAATGAGGAAGATCTACACCACTGTGCTGTTTG
P V Y W C R E D W P N Q E M R K I Y T T V L F
* * * * * 1120
CCAACATCTACCTGGCTCCCCTCTCCCTCATTGTGTCATCATGTATGGAAGGATTGGAATTTCACTCTTCAG
A N I Y L A P L S L I V I M Y G R I G I S L F R

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* * * * * 1190
GGCTGCAGTTCCTCACACAGGCAGGAAGAACCAGGAGCAGTGGCACGTGGTGTCCAGGAAGAAGCAGAAG
A A V P H T G R K N Q E Q W H V V S R K K Q K>
* * * * * 1260
ATCATTAAGATGCTCCTGATTGTGGCCCTGCTTTTTATTCTCTCATGGCTGCCCCTGTGGACTCTAATGA
I I K M L L I V A L L F I L S W L P L W T L M
* * * * * 1330
TGCTCTCAGACTACGCTGACCTTTCTCCAAATGAACTGCAGATCATCAACATCTACATCTACCCCTTTTGC
M L S D Y A D L S P N E L Q I I N I Y I Y P F A
* * * * * 1400
ACACTGGCTGGCATTTCGGCAACAGCAGTGTCAATCCCATCATTTATGGTTTCTTCAACGAGAATTTCCGC
H W L A F G N S S V N P I I Y G F F N E N F R
* * * * * 1470
CGTGGTTTCCAAGAAGCTTTCCAGCTCCAGCTCTGCCAAAAAGAGCAAAGCCTATGGAAGCTTATACCC
R G F Q E A F Q L Q L C Q K R A K P M E A Y T
* * * * * 1540
TAAAAGCTAAAAGCCATGTGCTCATAAACACATCTAATCAGCTTGTCAGGAATCTACATTTCAAACCC
L K A K S H V L I N T S N Q L V Q E S T F Q N P
* * * * * 1610
TCATGGGGAAACCTTGCTTTATAGGAAAAGTGCTGAAAACCCCAACAGGAATTAGTGATGGAAGAATTAA
H G E T L L Y R K S A E N P N R N>
* * * * * 1680
AAGAACTACTAACAGCAGTGAGATTTAAAAAGAGCTAGTGTGATAATCCTAACTCTACTACGCATTATA
* * * * * 1750
TATTTAAATCCATTGCTTTTTGTGGCTTTGCACTTCAAATTTTTCAAAGAATGTTCTAAATAAACATTT
* * * * * 1820
ACTGAAAGCCCTCTCTGGCAAAAAAATTAAAAATAAACAAAAATGGTCATAAGATCATAACAATCTTAT
* * * * * 1890
GTTGTATAAAAAATACGTAGAGTGACTTAGACATGTTTGCATGAATAAATATATTTCTAGAGAACAGTTAA
*
AAAAAAAAAAAA

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FIGURE 2

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*           *           *           *           *           *           *           *           *           *
70
ATGTCCACCATGAGCGAGAAATGGGACTCAAACCTCTTCAGAAAGCTGGAATCACATCTGGAGTGGAATG
M S T M S E K W D S N S S E S W N H I W S G N
*           *           *           *           *           *           *           *           *           *
140
ATACACAGCATCACTGGTATTTCAGATATCAACATTACCTATGTGAACTACTATCTCCACCAGCCCCAAGT
D T Q H H W Y S D I N I T Y V N Y Y L H Q P Q V
*           *           *           *           *           *           *           *           *           *
210
GGCAGCTGTCTTCATCAGCTCCTACCTCCTGATCTTTGTCTTGTGCATGGTGGGAAATACTGTCGTTTGC
A A V F I S S Y L L I F V L C M V G N T V V C
*           *           *           *           *           *           *           *           *           *
280
TTTATTGTGATAAGGAATAGACACATGCACACAGTCACTAATTTCTTGATCTTAAACCTTGCCATAAGTG
F I V I R N R H M H T V T N F L I L N L A I S
*           *           *           *           *           *           *           *           *           *
350
ATTTACTGGTTGGAATATTCTGTATGCCTATCACATTGCTGGACAACATCATAGCAGGATGGCCATTTCGG
D L L V G I F C M P I T L L D N I I A G W P F G
*           *           *           *           *           *           *           *           *           *
420
AAGCAGCATGTGCAAGATCAGTGGGCTGGTGCAAGGGATATCAGTTGCGGCTTCCGCTTTCACCTTGGTT
S S M C K I S G L V Q G I S V A A S V F T L V
*           *           *           *           *           *           *           *           *           *
490
GCAATAGCTGTGGACAGATTCGCTGTGTGGTCTACCCCTTTAAGCCAAAGCTCACTGTCAAGACAGCCT
A I A V D R F R C V V Y P F K P K L T V K T A
*           *           *           *           *           *           *           *           *           *
560
TTGTCACGATTGTGATCATCTGGGGCCTGGCCATCGCCATTATGACTCCATCTGCAATAATGTTACATGT
F V T I V I I W G L A I A I M T P S A I M L H V
*           *           *           *           *           *           *           *           *           *
630
ACAAGAAGAAAATACTACCGTGTGAGACTCAGCTCCCACAATAAAACCAGCACAGTCTACTGGTGTGCGG
Q E E K Y Y R V R L S S H N K T S T V Y W C R
*           *           *           *           *           *           *           *           *           *
700
GAGGACTGGCCAAGACACGAAATGAGGAGGATCTATACCACGGTGCTATTTGCCATCATCTATCTTGCTC
E D W P R H E M R R I Y T T V L F A I I Y L A
*           *           *           *           *           *           *           *           *           *
770
CTCTCTCACTCATTGTTATCATGTATGCAAGGATTGGGGCTTCCCTCTTCAAGACGGCAGCACACTGCAC
P L S L I V I M Y A R I G A S L F K T A A H C T
*           *           *           *           *           *           *           *           *           *
840
AGGCAAGCAGCGTCCAGTGCAGTGCATGTATCAAGAGAAACAGAAGGTCATCAAGATGCTGCTGACTGTG
G K Q R P V Q C M Y Q E K Q K V I K M L L T V
*           *           *           *           *           *           *           *           *           *
910
GCCCTCCTTTTCATCCTTTTCCTGGCTTCCCCTGTGGACCCTGATGATGCTCTCAGACTATACTGACCTGT
A L L F I L S W L P L W T L M M L S D Y T D L
*           *           *           *           *           *           *           *           *           *
980
CTCCTAACAACACTGCGTATCATCAACATCTACATCTACCCCTTTCGCCCCTGGCTCGCCTTCTGCAACAG
S P N K L R I I N I Y I Y P F A H W L A F C N S
*           *           *           *           *           *           *           *           *           *
1050
CAGTGTCAACCCTATTATTTATGGATTCTTTAATGAAAATTTTCGCAATGGTTTCCAAGATGCTTTCCAG

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S V N P I I Y G F F N E N F R N G F Q D A F Q
* * * * * 1120 *
ATCTGCCAAAAGAAAGCCAAGCCCCAGGAAGCCTATTCCCTGAGAGCGAAACGCAACATAGTCATAAACA
I C Q K K A K P Q E A Y S L R A K R N I V I N
* * * * * 1190 *
CATCGGGCCTGCTGGTGCAGGAACCGGTGTCTCAAACCCAGGTGGGGAAAAATTTGGGATGTGGAAAAAG
T S G L L V Q E P V S Q N P G G E N L G C G K S
* * * * *
TGCAGACAATCCACACAGGAATCCTTGATAGAGGAATG
A D N P H R N P *

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hy1p	1		MNSTLFSSQVENHSVHS	16
hy2p	1		MGPIGAEEADENQTVEEMKVEQYGPQT	26
hy7p	1		-MFIIMNEKWDNTNSSENWHPIWNVDIT	25
hy1p	17	NFSEKNAQLLAFENDDCHLPLAMIFTLLALAYGAVII	52	
hy2p	27	TPRGELVPDPEPELIDSTKLIEVQVVLLIAYCSILL	62	
hy7p	26	KHHLYS DINITYVNYYLHQP-QMAAIFIIISYFLIFF	60	
hy1p	53	LGVSGNLAIIILIKQKEMRNVTNILIVNLISFSDLL	88	
hy2p	63	LGVIGNSLVIHVVIKF KSMRTVTNF FIANLAVADLL	98	
hy7p	61	LCMMGN TVMC FIVMRNKHMHTVTNFLFILNLAI SDLL	96	
hy1p	89	VAIMCLPFTF VYTLM DHWVFEGEAMCKLNPFVQC VSI	124	
hy2p	99	VNTLCCLPFTLT TYTLMGEWKMPVLCHLVPIYAQGLAV	134	
hy7p	97	VGIFC MPITLLDNIIAGWPFGNTMCKISGLVQG ISV	132	
hy1p	125	TVSIESLVLI AVERHQ LIINPRGWRPNNRHAYVGI A	160	
hy2p	135	QVSTITLT VI ALDRHRGIVYHLESKISKRISFLIIG	170	
hy7p	133	AASVFETLV AI AVDRFQC VVYPFKPKLT IKT AFV IIM	168	
hy1p	161	VIWVLA VASSLP FLTYQVM TDEPFQNMTLD - - - AYK	193	
hy2p	171	LAWGISALLASPLAI - - - - FREYSL IEIIPDF	198	
hy7p	169	I IWVLA ITIMS PSAVMLHVQE EKYYRVRLINS QNKTS	204	
hy1p	194	DKYVC FDQFP SDSHRLS YTTLLL V - - - LQYFG PLCF	226	
hy2p	199	EIVACTEK WPGE EKS IYGTVYSSL SLL ILYL VLPLGI	234	
hy7p	205	PVYWCRE DWPNQ EMRK IYTTV LFANI - - - YLAPLSL	237	
hy1p	227	IFICYFKI YIRLRKRNN - - - MMDKMRDNKYRSSETK	259	
hy2p	235	ISFSYTRIWS KLKNHVSP - - GAANDHYHQRRQKTK	268	
hy7p	238	IVIMYGRIGISLFRAAVPH TG RKNQE QWHVVS RKKQ	273	
hy1p	260	RINIMLLS IVVAFAV CWLPLTI FNTVFDW - - NHQ II	293	
hy2p	269	- - - MLVCVVV VF AVSWLPLHA FQLAVDI - - DSQVL	298	
hy7p	274	KIKIMLLI VALLFIL SWLPLWT LMMLS DYADL SPNE	309	
hy1p	294	ATCNHN LLFLLCH LTAMIST CVNP IFYGF LNKNFQR	329	
hy2p	299	DLKEYKL IFTVFHI IAM CSTF ANPLL YGWMNSNYRK	334	
hy7p	310	LQIINIYIYPFAHWLAF GNSS VNPI IYGF FN ENFRR	345	
hy1p	330	DLQFFF NFCDFRSRDDDY ETIAMSTMHTDVSKTSLK	365	
hy2p	335	AFLSAFRCEQRLDAIH SEVS VT FKAKKNLEVRKNSG	370	
hy7p	346	GFEAEFQLQLCQKRAKPM EAYTLKAKSHVLINTSNQ	381	
hy1p	366	QASPVAFFKKINNND DNEKI	384	
hy2p	371	PNDSFTEATNV	381	
hy7p	382	LVQUESTFQNP HGETLLYRKSAENPNRN	408	

Human-Mouse NPY Y7 Receptor Alignment

hy7	1	M	F	I	M	N	E	K	W	D	T	N	S	S	E	N	W	H	P	I	W	N	V	N	D	T	K	H	H	L	Y	S	D	I	N	I	T	Y	V	38
mY7	1	M	S	T	M	S	E	K	W	D	S	N	S	S	E	S	W	N	H	I	W	S	G	N	D	T	Q	H	H	W	Y	S	D	I	N	I	T	Y	V	38
hy7	39	N	Y	Y	L	H	Q	P	Q	V	A	A	I	F	I	I	S	Y	F	L	I	F	F	L	C	M	M	G	N	T	V	V	C	F	I	V	M	R	N	76
mY7	39	N	Y	Y	L	H	Q	P	Q	V	A	A	V	F	I	I	S	Y	L	L	I	F	V	L	C	M	V	G	N	T	V	V	C	F	I	V	I	R	N	76
hy7	77	K	H	M	H	T	V	T	N	L	F	I	L	N	L	A	I	S	D	L	L	V	G	I	F	C	M	P	I	T	L	L	D	N	I	I	A	G	W	114
mY7	77	R	H	M	H	T	V	T	N	F	L	I	L	N	L	A	I	S	D	L	L	V	G	I	F	C	M	P	I	T	L	L	D	N	I	I	A	G	W	114
hy7	115	P	F	G	N	T	M	C	K	I	S	G	L	V	Q	G	I	S	V	A	A	S	V	F	T	L	V	A	I	A	V	D	R	F	Q	C	V	V	Y	152
mY7	115	P	F	G	S	S	M	C	K	I	S	G	L	V	Q	G	I	S	V	A	A	S	V	F	T	L	V	A	I	A	V	D	R	F	R	C	V	V	Y	152
hy7	153	P	F	K	P	K	L	T	I	K	T	A	F	V	I	I	M	I	I	W	V	L	A	I	T	I	M	S	P	S	A	V	M	L	H	V	Q	E	E	190
mY7	153	P	F	K	P	K	L	T	V	K	T	A	F	V	T	I	V	I	I	W	G	L	A	I	A	I	M	T	P	S	A	I	M	L	H	V	Q	E	E	190
hy7	191	K	Y	Y	R	V	R	L	N	S	Q	N	K	T	S	P	V	Y	W	C	R	E	D	W	P	N	Q	E	M	R	K	I	Y	T	T	V	L	F	A	228
mY7	191	K	Y	Y	R	V	R	L	S	S	H	N	K	T	S	T	V	Y	W	C	R	E	D	W	P	R	H	E	M	R	R	I	Y	T	T	V	L	F	A	228
hy7	229	N	I	Y	L	A	P	L	S	L	I	V	I	M	Y	G	R	I	G	I	S	L	F	R	A	A	V	P	H	T	G	R	K	N	Q	E	Q	W	H	266
mY7	229	I	I	Y	L	A	P	L	S	L	I	V	I	M	Y	A	R	I	G	A	S	L	F	K	T	A	A	H	C	T	G	-	-	K	Q	R	P	V	Q	264
hy7	267	V	V	S	R	K	K	Q	K	I	I	K	M	L	L	I	V	A	L	L	F	I	L	S	W	L	P	L	W	T	L	M	M	L	S	D	Y	A	D	304
mY7	265	C	M	Y	Q	E	K	Q	K	V	I	K	M	L	L	T	V	A	L	L	F	I	L	S	W	L	P	L	W	T	L	M	M	L	S	D	Y	T	D	302
hy7	305	L	S	P	N	E	L	Q	I	I	N	I	Y	I	Y	P	F	A	H	W	L	A	F	G	N	S	S	V	N	P	I	I	Y	G	F	F	N	E	N	342
mY7	303	L	S	P	N	K	L	R	I	I	N	I	Y	I	Y	P	F	A	H	W	L	A	F	C	N	S	S	V	N	P	I	I	Y	G	F	F	N	E	N	340
hy7	343	F	R	R	G	F	Q	E	A	F	Q	L	Q	L	C	Q	K	R	A	K	P	M	E	A	Y	T	L	K	A	K	S	H	V	L	I	N	T	S	N	380
mY7	341	F	R	N	G	F	Q	D	A	F	Q	I	-	-	C	Q	K	K	A	K	P	Q	E	A	Y	S	L	R	A	K	R	N	I	V	I	N	T	S	G	376
hy7	381	Q	L	V	Q	E	S	T	F	Q	N	P	H	G	E	T	L	L	Y	R	K	S	A	E	N	P	N	R	N										408	
mY7	377	L	L	V	Q	E	P	V	S	Q	N	P	G	G	E	N	L	G	C	G	K	S	A	D	N	P	H	R	N	P									405	

FIGURE 4

Mouse NPY-Y7 Gene

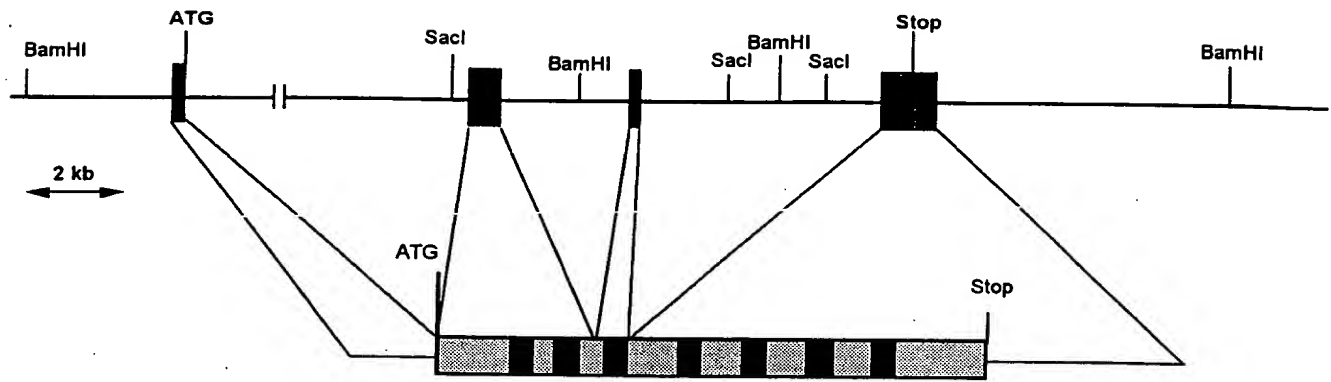


FIGURE 5

FIGURE 6

[125I]PYY (50pM)

Inhibition of human [125I]PYY binding with various NPY-related peptides on human Y7 membranes

- PYY
- ▲ NPY
- ▼ [2-36]NPY
- ◆ [13-36]PYY
- Leu31Pro34NPY
- PP

